

Behavioral Response of Dolphins to Signals Simulating Mid-Frequency Sonar

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LONG-TERM GOALS

The ultimate goal of the behavioral response study, which includes dolphin and sea lion subjects, is to provide data on the response of marine mammals to the exposure of sonar-like signals across a range of receive levels (RL; dB re 1 μ Pa). The study is designed to provide data for risk functions used by the United States Navy in predictions of harassment as defined under the Marine Mammal Protection Act.

OBJECTIVES

The objectives of this effort are to:

- 1a) Determine the occurrence and magnitude of behavioral responses observed in bottlenose dolphins exposed to variable levels of mid-frequency (~3 kHz) sounds similar to tactical sonar signals; 1b)

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apply a scoring analysis to qualitative descriptors of the observed responses; and 1c) calculate a behavioral dose-response function (DRF) for delphinid and otariid species exposed to mid-frequency signals.

2) Record heart rate on a subset of the dolphins and sea lions involved in the behavioral response study to determine whether an acute stress response occurs in response to the mid-frequency sound exposure. The response will be related to the exposure characteristics of the sound received by the animal to determine if there is a relationship between the sound exposure and the magnitude and duration of the response.

3) Collect blood samples from the dolphins prior to, immediately following, and for several days to a week following the sound exposure. Blood collections will be analyzed for cortisol and aldosterone and inspected for parallelism to determine whether a prolonged stress response is triggered by the sound exposure event. In addition, samples will be analyzed for epinephrine to determine if an acute stress response occurs following sound exposure.

APPROACH

A group of dolphins ($n = 30$) and sea lions ($n = 15$) will be trained on a modified “A to B” task. The subject will be cued by a trainer to leave a station (A), travel to another station (B) and touch a paddle, and then return to the original station (A) for a fixed fish reward; hereafter termed the ABA task. Subjects will be trained on the ABA task until there is a 100% completion percentage across a 10 trial block. Upon meeting the nominal performance criterion, the subjects will perform a 10 trial control session followed by a 10 trial sound exposure session.

Control and exposure sessions will be performed in a 30×60 ft floating pen with an underwater sound projector, designated as T, placed behind station B. An instrumentation hut will be placed to the side of the pen. Each trial within the control and exposure sessions will be limited to 30 s. The reward for completing each trial, control or exposure, will be a fixed number of fish equal to ~1% of the weight of the daily allotment of fish for that subject. For the control session, each subject will perform 10 trials of the ABA task without acoustic exposure. A second session will then be conducted several minutes later, during which the subject will receive an acoustic exposure at a predetermined point along its trajectory within the floating pen. On each trial, the exposure will be performed each time the subject passes the exposure point on its way to station B. Acoustic exposures will consist of a 0.5 s upward FM sweep (center frequency ~3250 Hz) followed by a 0.5 s CW (~3450 Hz). Subjects will be naïve to the exposure and only one trial block will be used for each subject.

Exposure levels will be consistent for each individual (i.e. all trials within a session will be at the same exposure level), but will be varied from individual to individual in order to provide a range of exposure levels. For dolphin tests, exposure levels will range from ~115 to 185 dB re: 1 μ Pa (rms) and will be varied in 10-15 dB steps. This design permits 5 individual dolphins to be tested at each exposure level. For sea lions the exposure range will be from 125 to 185 dB; the range of exposure levels and number of subjects per exposure level will be reduced to accommodate the smaller number of available animals. Dolphins and sea lions will be randomly assigned to an exposure level category for their assigned trial block and the individuals conducting the tests will be blind to the assigned exposure levels until the day of the test.

The floating pen will be outfitted with overhead supports for positioning video cameras. Video recordings of all sessions will be collected and stored to a digital video recorder for later analysis. Known points along the pen will be used to demarcate the point at which sound exposure should occur, i.e. the acoustic exposure will be triggered by an observer when the dolphin or sea lion passes the trigger point.

A trainer will be positioned at station A and another at station B. The session controller will be positioned in the control hut and will have direct visual access to the pen as well as video and acoustic surveillance. The trainers and session operator will be in vocal communication via wireless intercoms and communications will be mixed with video signals and stored to DVR. The trainer at station A will be responsible for providing cues to the animal to begin the ABA task and for rewarding the completion of the task. No other interaction with the animal is permitted for the trainer at station A. Both trainers will be responsible for reporting behavioral reactions. The session operator will be responsible for controlling the pace of the session – keeping track of trial times, triggering exposures, directing trial starts and animal recalls.

Monitoring hydrophones will be used to record underwater sounds produced by the animals. Underwater recordings will be mixed down with a duplicate of the video recordings in order to obtain aligned acoustic and visual events. Audio/video files will be used for post-session analysis of behavioral responses.

The suite of behavioral responses anticipated to occur following sound exposure will be *a priori* scored for severity to avoid potential bias in severity scoring after data collection. Severity scores will be determined by submitting the list of behavioral responses to a group of marine mammal scientists who will independently assign severity scores to the behavioral responses. The behavioral responses of individual animals will be recorded during each sound exposure trial (10 exposure trials in a session) and during each baseline trial. At the completion of the study the severity scores will be applied to the observed behavioral responses and the results will be used to determine the dose-response relationship between sound exposure and the severity of behavioral reactions.

Blood samples will be collected approximately one week prior, immediately following the exposure session, and approximately one week after testing. Samples will be analyzed via radioimmunoassay for levels of corticosteroid hormones (cortisol and aldosterone) and epinephrine. Levels will be compared between categories and related to exposure level to determine whether acute or chronic stress responses resulted from the exposure. In addition, a subset of the animals that are trained to wear a harness will be fitted with an ECG system to record heart rate during control and experimental trials. As with the catecholamine analysis, variations in heart rate will be assessed for changes associated with the sound exposure.

Dorian Houser will be primarily responsible for conducting the study, overseeing the acoustic and video analysis, performing the RIA analyses, and maintaining the overall project management. James Finneran will contribute with engineering support and acoustic recording setups. Laura Yeates will be responsible for the heart rate recordings of the animals.

WORK COMPLETED

Twenty-four scientists were invited to participate in the severity scoring of potential behavioral responses. Of these, 14 individuals completed and returned severity scores for the potential behavioral responses.

The testing of all bottlenose dolphin and sea lion subjects was completed in January of 2011. The behavioral reactions of all dolphin and sea lion sessions were independently assessed by two individuals that were blind to the exposure conditions on each trial. Severity scores were applied to the observed behavioral reactions. The resultant severity scores by trial were analyzed with an asymmetrical dose function; overall scores were utilized for one analysis, and another was conducted in which only the abandonment of the ABA behavior was scored.

Eighty-nine of 90 blood samples attempted on the bottlenose dolphins were collected and all samples were analyzed for stress hormones. Heart rate collections were attempted on ~1/3 of the dolphin subjects and preliminary analyses of the heart rate data was completed.

RESULTS

Dolphins:

An intra-class correlation one-way random effects analysis of subject matter expert severity scores demonstrated considerable variability on the severity ranking of individual behaviors (ICC single measures = 0.367). As a result, average scores were similar across many behavioral responses and application of the raw summed scores for individual trials made the fitting of any function to the response severity scores difficult and all fits produced large error estimates. However, an asymmetric function was fit to the data utilizing only task abandonment as a binary response variable; it became apparent that a symmetric dose response function was overly constrained and did not fit the distribution of data well. Asymmetric dose response functions for several trials are presented in Figure 1. The r^2 for dose response function fits ranged from 0.53-0.65 and the midpoint of the function (Log EC₅₀) ranged from 174.4-180 dB RL. However, abandonment was noticed at received levels (RL) as low as 130 dB.

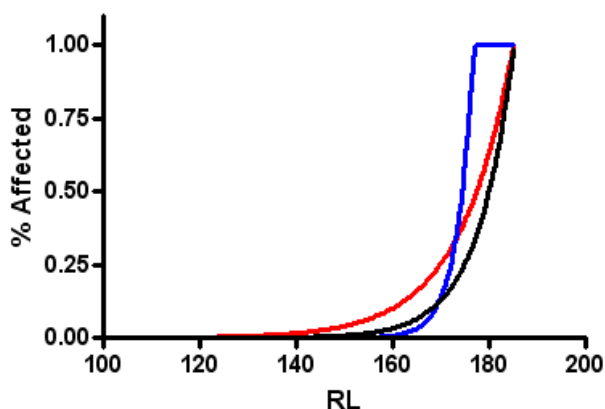


Figure 1. Dose response functions are presented for the first (red), fifth (blue), and tenth (black) trials of the ten trial exposure sessions. The figure shows the percentage of the population that abandoned the trained behavior as a function of the received sound pressure level.

There was no significant relationship between behavioral response or exposure level to any of the stress hormones measured in the dolphins. Similarly, no significant increase in heart rate was observed following a sound exposure, even though behavioral responses were in some cases profound (e.g. immediate abandonment of behaviors, rapid and erratic swimming, tail slaps, chuffs).

Sea lions:

Sea lion behavioral reactions were more varied than that of the dolphins across the range of exposure levels. Using only abandonment of the ABA behavior as a response variable, convergence of an asymmetric dose response model was not achieved for most trials. However, the 10th trial converged ($r^2=0.54$, Log EC₅₀=156.5), and demonstrated a greater probability of responding at lower received levels than was observed in the dolphins. The distribution of responses was affected in part by the age of the animals tested; all of the animals that responded to received levels less than 155 dB SPL were two years of age or less.

IMPACT/APPLICATIONS

The controlled exposure study demonstrated that a symmetrical dose response function is unlikely to adequately fit the distribution of responses within exposed subsets of a marine mammal population. For dolphins, above a certain received level, every dolphin abandoned the behavior. Below this level, the responses were more variable and dictated by context. In this study, since context was kept the same for all animals, the variability reflected individual animal sensitivity to novel acoustic stimuli. Although the dose response functions derived here may not directly reflect that of a wild dolphin population, it is possible that the pattern of predictable abandonment above a certain received level and a more variable, context-dependent response below a certain received level will be observed in wild populations. The pattern is less clear in sea lions. Although they demonstrated behavior abandonment at lower received levels, the distribution of responses was heavily influenced by the youngest animals. A similar distribution of responses by age and experience can probably be expected from wild populations as well.

A lack of any physiological indicator of an acute stress response questions whether measurement of these indicators (heart rate, epinephrine) will be adequate for assessing a response to acoustic exposures in dolphins. It may be that the startle response, as has been defined for terrestrial mammals, does not involve variations in heart rate and catecholamine release in diving marine mammals. Indeed, it may be that the need to control oxygen while at depth, where animals are prevented from replenishing oxygen stores, has resulted in diving marine mammals decoupling heart rate and catecholamine release from the startle response. For a marine mammal, evolutionary pressures in the form of near-surface predators may have produced a physiological response that maintains oxygen management and permits the diving marine mammal to stay at depth longer (i.e. to avoid the threat).

RELATED PROJECTS

Project #: N000141110432

Title: Biomarkers to Assess Possible Biological Effects on Reproductive Potential, Immune Function, and Energetic Fitness of Bottlenose Dolphins Exposed to Sounds Consistent with Naval Sonars

Blood samples collected as part of Project# N0001409WX20853, "Behavioral Response of Dolphins to Signals Simulating Mid-Frequency Sonar," were sent to the Mote Marine Laboratory for assessment of stress markers. The project seeks to determine if novel stress markers can be identified that show a response to acoustic exposure.